IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: Yong Soo Chong
Timothy Tully, et al.	Art Unit:1617
Application Serial No.: 09/927,914	Confirmation No: 5180
Filed: August 10, 2001	Attorney's Docket No.43373-0008
For: **Augmented Cognitive Training**	**Customer No. 25213**

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION OF TIMOTHY TULLY, Ph.D UNDER 37 C.F.R. § 1.132

I, Timothy Tully, Ph.D. declare and say as follows:

1. I was a Professor at Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724 from September 1, 1991 until May 31, 2007. I am Chief Science Officer of Dart Neuroscience LLC as of June 1, 2007.

2. I have been Acting Chief Scientific Officer at Helicon Therapeutics, Inc. Farmingdale, NY 11735 since July 1, 1997. My scientific Curriculum Vitae, including my list of publications, is attached to and forms part of this Declaration (Exhibit A).

3. I have been involved in supervising and analyzing the effect of phosphodiesterase inhibitors and training on performance gain during treatment for a cognitive deficit as set forth in the above referenced patent application.

4. I am aware that some of the claims in the above captioned patent application have been rejected under 35 U.S.C. § 112 as allegedly lacking enablement. My understanding is that

the rejection is based, at least partially, on the assertion that it is unpredictable as to whether administration of any phosphodiesterase inhibitor would result in performance gain during treatment of a cognitive deficit associated with a central nervous system disorder.

5. To the contrary, however, it is my considered scientific opinion that administration of any augmenting agent which enhances CREB pathway function by inhibiting a phosphodiesterase in combination with cognitive training would result in performance gain during treatment of a cognitive deficit associated with central nervous system disorder.

6. In support of the conclusion made in paragraph 5 above, I offer the following evidence attached as Exhibit B. In this experiment, the efficacy of the phosphodieterase inhibitor HT0712 in promoting rehabilitation dependent motor recovery and enhancing functional restoration within the motor cortex following cortical ischemia was examined.

7. The experiment described in Exhibit B shows that recovery which is associated with a reinstatement and reorganization of function within residual tissue, can be upregulated via the inhibition of phosphodiesterase in combination with rehabilitative training. With the exception of animals receiving 0.30 mg/kg, all animals receiving the phosphodiesterase inhibitor HT0712 in combination with motor rehabilitation had significantly larger motor maps and better post-stroke reaching performance than vehicle injected controls. The results of the experiment indicate that the phosphodiesterase inhibitor HT0712 contributes to recovery of motor function by augmenting the restoration of cortical function that occurs during rehabilitation.

8. Furthermore, the performance gain during treatment of a cognitive deficit associated with central nervous system disorder can be achieved with any augmenting agent which enhances CREB pathway function by inhibiting a phosphodieterase. Although there are various augmenting agents which may enhance CREB pathway function by inhibiting a phosphodiesterase by a number of different mechanisms, signaling through phosphodiesterase is inhibited, regardless of the manner of inhibition. Thus, the CREB pathway function will be enhanced. Accordingly, ultimately the common mechanism of action of all phosphodiesterase inhibitors is inhibiting the ability of phosphodiesterases to inhibit the CREB pathway function.

9. It necessarily follows from paragraphs 6, 7 and 8 that administration of any augmenting agent which enhances CREB function by inhibiting a phosphodiesterase would result in performance gain during treatment of a cognitive deficit associated with central nervous system disorder.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Signed: Timothy J. Tully

Timothy Tully, Ph.D.

Date: 7/30/07

7/30/07 11:16 AM ()

EXHIBIT A

CURRICULUM VITAE

TIM TULLY

Chief Science Officer	2007	Dart Neuroscience, LLC
Division Head, NeuroGenetics	2006	Cold Spring Harbor Laboratory
Adjunct Professor	2004	Institute of Brain Research National Tsing Hua University, Taiwan
St. Giles Foundation Professor of Neuroscience	2003	Cold Spring Harbor Laboratory
Guest Professor	2002	Department of Biological Sciences Tsinghua University, China
Visiting Scholar	2002	Department of Neurobiology, SUNY Stony Brook
Professor	1995	Cold Spring Harbor Laboratory
Affiliate Professor	1994	Genetics Program, SUNY Stony Brook
Affiliate Professor	1994	Neuroscience Program Cornell University Medical College
Visiting Scholar	1994	Biology Department, New York University
Associate Professor	1991	Cold Spring Harbor Laboratory
Assistant Professor	1987	Biology Department, Brandeis University
Research Associate	1985	Molecular Genetics Massachusetts Institute of Technology
Postdoctoral Fellow	1981	Neurogenetics, Princeton University
Doctor of Philosophy	1981	Genetics, University of Illinois
Bachelor(s) of Science	1976	Biology & Psychology, University of Illinois

Honors, Awards and Professional Activities

Awards Committee, International Behavioral & Neural Genetics Society, 2003 -
Scientific Advisory Board, Joekai Biotech Co., Ltd, 2003 -
Scientific Review Board, Institute for the Study of Aging, 2001 -
Editorial Advisory Board, *Genes, Brain and Behavior*, 2001 -
Acting Chief Scientific Officer, Helicon Therapeutics, Inc., 2001 -
Selection Committee, Lindsley Prize, Society for Neuroscience, 2000 - 2005
Decade of the Brain Award, American Academy of Neurology, 1999
Board of Trustees, The Swartz Foundation, 1997 - 2000
Board of Directors, Helicon Therapeutics, Inc., 1997-
John A. Hartford Foundation Grantee, Cold Spring Harbor Laboratory, 1997-2000
Editorial Advisory Board, *Learning & Memory*, 1995 -
Editorial Advisory Board, *Behavior Genetics*, 1992 -
John Merck Scholarship in the Biology of Developmental Disabilities in Children, 1990 - 1994
Editorial Advisory Board, *Behavioral Neuroscience*, 1989 - 2001
Associate Editor, *Behavior Genetics*, 1987 - 1992
McKnight Scholars Award in Neuroscience, Brandeis University, 1987 - 1990
NIH Postdoctoral Fellow, Princeton University, 1981 - 1985
NIMH Predoctoral Trainee, University of Illinois, 1978 - 1981

Membership in Professional Societies

Society for Neuroscience
Genetics Society of America
American Psychological Association
International Society for Neuroethology
International Behavioural and Neural Genetics Society

Publications (Citations)

Matsuno M., Tully T. and M. Saitoe (2007) The *Drosophila* cell-adhesion molecule Klingon is required for long-term memory and is regulated by *Notch.* (submitted).

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Chiang A.-S., Blum A., Barditch J., Chen Y.-H., Chiu S.-L., Regulski M., Armstrong J.D., Tully T. and J. Dubnau (2004) *radish* encodes a phospholipase-A2 and defines a neural circuit involved in anesthesia resistant memory. *Current Biology 14*: 1-20.

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Yin J.C.P., Del Vecchio M., Zhou H. & T. Tully (1995) CREB as a memory modulator: induced expression of an *hsp-dCREB2-a* activator isoform enhances the formation of long-term memory. *Cell 81*: 107-115. **(272)**

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Dubnau J., Chiang, A.-S. & T. Tully (2003) Neural substrates of memory: from synapse to system. *Journal of Neurobiology54*: 238-253. **(5)**

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EXHIBIT B

EXHIBIT

Motor impairments represent the most common form of disability resulting from stroke. Depending on the severity of the impairments, motor rehabilitation can result in significant improvements in motor function over time. The efficacy of the phosphodiesterase inhibitor HT0712 ((3S, 5S)-5-(3-cyclopentyloxy-4-methoxy-phenyl)-3-(3-methyl-benzyl)-piperidin-2-one; also known as IPL 455,903)) in promoting rehabilitation-dependent motor recovery and enhancing functional restoration within the motor cortex following cortical ischemia was examined.

Materials and Methods

Subjects: Forty-two adult (90 days) male Long-Evans hooded rats (350-420g) were group housed (2 animals/cage) in standard laboratory cages on a 12:12 hour light dark cycle throughout the experiment.

Reach Training: Over the course of several days, all animals were placed on a restricted diet until they reached 90% of their original body weight. A brief period of pretraining was then given to familiarize the rats with the reaching task. This involved placing the animals into test cages (10 X 18 X 10 cm) with floors constructed of 2 mm bars, nine mm apart edge to edge. A four cm wide and 5 cm deep tray filled with food pellets (45 mg; Bioserv) was mounted on the front of the cage. The rats were required to reach outside the cage and retrieve pellets from the tray. All rats remained in pretraining until they had successfully retrieved 10 pellets (approximately 1 hour/day for 2 days). After pretraining, the rats were placed into a Plexiglas cage (11 cm X 40 cm X 40 cm) with a 1 cm slot located at the front of the cage. Animals were trained for 20 minutes each day to reach through the slot and retrieve food pellets from a table outside the cage. Rats were permitted to use either limb and the preferred limb was noted for each animal. Each session was videotaped and later used to assess reaching performance. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The percentage of successful reaches [(# successful retrievals/the total # of reaches) x 100] was then calculated. Animals were trained for

approximately 2 weeks on this task to establish a baseline measure of motor performance. Baseline was defined as the average accuracy across the three final days of training. Post stroke performance was expressed as a percentage of the baseline performance.

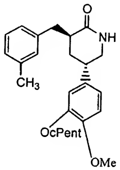
Electrophysiological Mapping: Within 2 days of the final training session, standard intracortical microstimulation (ICMS) techniques were used to generate detailed maps of forelimb regions of the motor cortex contralateral to the trained forelimb. Prior to surgery animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.). Animals received low levels of isofluorane (0.15%) and supplemental doses of ketamine (20 mg/kg i.p.) as needed. Under sterile conditions, a craniotomy was performed over the motor cortex contralateral to the trained paw of each animal. To prevent edema, a small puncture was made in the cisterna magna prior to removing the skull and dura. The exposed cortex was then covered in warm saline (37°C). A digital image of the cortical surface was taken and a 375 □m grid was superimposed onto the image. A glass microelectrode (controlled by a hydraulic microdrive) was used to make systematic penetrations across the cortex using the cortical surface image and grid as a guide. At each penetration site, the electrode was lowered to approximately 1550 μm (corresponding to cortical layer V). Stimulation consisted of 13, 200 μs cathodal pulses delivered at 350 Hz from an electrically isolated stimulation circuit. Animals were maintained in a prone position with the limb consistently supported. Sites where no movement was detected at ≤ 60 μA were recorded as unresponsive. Forelimb movements were classified as either distal (wrist/digit) or proximal (elbow/shoulder) and representational maps were generated from the pattern of electrode penetrations. The caudal forelimb area (CFA) was defined by a medial boundary of vibrissa representations, a lateral and caudal boundary of non response sites and a rostral boundary of head and neck representations. An image analysis program (CANVAS v. 3.5) was used to calculate the area extent of the caudal forelimb area (CFA).

Focal Infarction: Focal ischemic infarcts were created within caudal forelimb area via bipolar electrocoagulation of the surface vasculature. The infarct targeted primarily the distal forelimb representations but in some cases included small regions of proximal representations. The coagulated vessels included fine arterial and venous capillaries as well as larger vessels but specifically avoided any bypassing arteries supplying other cortical areas. Coagulation was

continued until all vessels within the targeted area were no longer visible and the tissue appeared white.

Motor Rehabilitation: Within three days of the initial mapping and infarction procedure, all animals were placed into a motor rehabilitation program that consisted of being trained daily for 15 minutes on the skilled reaching task described above for 10 days. Animals were also randomly assigned to one of five doses of HT0712: Vehicle (n=8), 0.10 mg/kg (n=7), 0.15mg/kg (n=8), 0.30 mg/kg (n=9), and 0.10 mg/kg given twice per day. All animals received injections 20 minutes prior to the daily training session with the exception of one group of 0.10 mg/kg animals that received a second injection 3 hours after training. The sessions were video taped and reaching accuracy was assessed as described above.

HT0712 has the following formula:



wherein "Me" means "methyl" and "cPent" means "cyclopentyl". HT0712 can be prepared using the methodology provided in U.S. Patent No. 6,458,829B1.

Assessing Cortical Dysfunction: Within one day of the final training session, ICMS was again used to generate a second map of the caudal forelimb area (CFA) contralateral to the trained forelimb. Prior to surgery animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.), receiving xylazine (0.02 mg/kg i.m.) and ketamine (20 mg/kg i.p.) as needed. Further, animals were placed on isoflurane (.15%, 1.5% O_2) when needed. The dental polymer, gel film and gel foam were removed and the exposed cortex covered in warm silicon oil. Mapping procedures were identical to those used in the initial mapping.

Results

Skilled Reaching: A repeated measures Analysis of variance (ANOVA) with DAY as a within subjects factor and CONDITION as a between subject factor revealed a significant DAY X CONDITION interaction [$F(9,36) = 1.72$; $p<0.05$) on reaching performance. Subsequent multiple comparisons (Fishers PLSD; $p <0.05$) showed the 0.15 mg/kg and 0.10 twice per day HT0712 injected animals to have a significantly higher reaching accuracy than all other groups during the later stages of training. The 0.10 mg/kg animals had significantly better reaching accuracies than the Vehicle and 0.30 mg/kg HT0712 animals.

Map Area: An analysis of variance with CONDITION as a between subject factor revealed a no significant effect of CONDITION on Map 1 area [$F (4,36) = 1.1$ $p>0.05$]. A significant main effect of CONDITION on Map 2 [$F(4,36) = 6.5$; $p<0.05$]. Subsequent multiple comparisons (*Fishers PLSD; $p<0.05$) showed the 0.10 mg/kg had significantly 0.10 mg/kg twice/day and the 0.15 mg/kg all had significantly larger Map 2 than the 0.30 mg/kg and Vehicle injected animals.

Discussion

Functional impairments following brain injury are due to both the loss of tissue within the damaged area and concomitant dysfunction within other brain area. The results of the present study show that recovery, which is associated with a reinstatement and reorganization of function within residual tissue, can be upregulated via the inhibition of phosphodiesterase in combination with rehabilitative training. Further, the expansion of movement representations within peri-infarct areas was accompanied by enhanced motor recovery. The increase in peri-infarct motor map area represents the restoration of cortical circuitry that is augmented through the upregulation of cAMP. The results of the present study demonstrate a dose dependent increase in motor recovery and enhanced functional restoration within motor cortex. With the exception of the animals receiving 0.30 mg/kg, all animals receiving HT0712 in combination with motor

rehabilitation had significantly larger motor maps and better post-stroke reaching performance than vehicle injected controls.

The results indicate that HT0712 contributes to recovery of motor function by augmenting the restoration of cortical function that occurs during rehabilitation. Specifically, HT0712 may act to facilitate synaptic strengthening and the reinstatement of the cortical circuitry required to support both the motor maps and skilled motor behavior.